QUENCHING OF CHLOROPHYLL-a FLUORESCENCE BY REDUCED PMS

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1. Introduction

A number of authors [1–4] have reported that the chl-a fluorescence yield of chloroplasts and intact algal cells can be quenched in the light when small concentrations of PMS were added to the reaction. This 'fluorescence lowering' occurred in the presence of DCMU and with high light intensities. Since PMS is a powerful catalyst for cyclic photophosphorylation and since the effect was abolished by uncouplers of photophosphorylation such as atebrine and CCCP, it has been suggested that the quenching reflected the development of a high energy intermediate related to the synthesis of ATP.

These interpretations contrast with observations (unpublished results) that the fluorescence quench still occurs with a number of other uncouplers such as gramicidin and NH_4Cl.

We show here, that the chl-a fluorescence of chloroplasts can be quenched to a high extent when PMS is reduced either chemically or enzymically.

2. Methods

Chloroplasts were prepared from peas according to Rurainski et al. [5]. They were incubated in a Thunberg cuvette for at least 2 min in the dark in a reaction medium containing 100 mM sucrose, 20 mM Tricine pH 8.0, 10 mM NaCl, 5mM $MgCl_2$, 40 μM DCMU and 10 mM glucose. After this time, additions as described in the legends to the figures were made from the side arm.

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PMS reduction was followed in a Zeiss spectrophotometer PMQ 2. For fluorescence measurements a non-commercial spectrophotometer was used. Chl-a fluorescence was excited by light from a 150 W halogen light source which was filtered through a narrow banded interference filter (Schott) with a peak transmission at 595 nm and a half band width of 10 nm and passed through a Compur photoshutter which opened and closed within 3 msec, respectively. Light intensity was 34 kerg/cm^2 sec. The photomultiplier was situated rectangular to the actinic light beam and was protected against scattered actinic light by a combination of a broad band interference filter (Balzer k-7) and a narrow banded interference filter (Schott) with peak transmission at 687 nm and a half band width of 10 nm. The signal was temporarily stored in a Nicolet signal averager having 1024 sampling channels and plotted on a x/y-recorder.

3. Results and discussion

The reaction of glucose and oxygen via glucose oxidase [6] is a common means to produce anaerobiosis in a reaction vessel. Under the conditions used here all oxygen was depleted after 5 min of incubation. The chl-a fluorescence level in the presence of DCMU but without PMS was not significantly altered by anaerobiosis (fig.1, curves a and b). Neither did addition of 5 mM ascorbate to the reaction mixture change the fluorescence level to an appreciable extent. Only when 20 μM PMS was present during the incubation with glucose oxidase or ascorbate, a decreased chl-a fluorescence level was obtained which was approximately 20% of the original level (fig.1, curve d). Thus, we can exclude from these data that the fluorescence

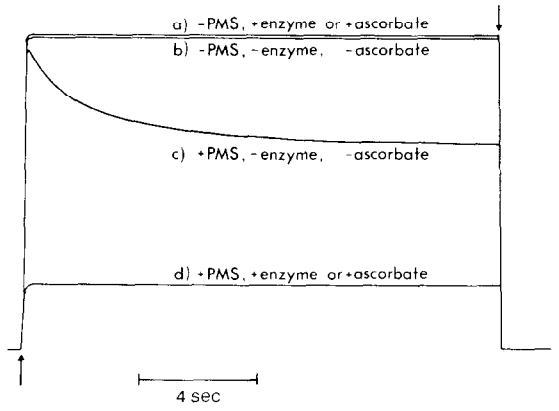

Fig.1. Time course of fluorescence with or without PMS, glucose oxidase or ascorbate. Chloroplasts equivalent to 8 μg chlorophyll ml were incubated for 5 min in reaction medium (see Methods) which additionally contained, where indicated, 20 μM PMS, about 1 unit/ml glucose oxidase and 5 mM ascorbate. The upward and downward arrows refer to light on and light off, respectively.

decrease is due to anaerobiosis, the effect of H_2O_2 which is produced by the glucose oxidase reaction or the action of ascorbate.

The low fluorescence level in fig.1, curve d could be reproducibly obtained only after an incubation time of at least 5 min. At shorter times, an intermediate level was observed, which further decreased during actinic illumination. The initial time course of this process is demonstrated for the glucose/glucose oxidase system in fig.2 using different concentrations of PMS.

At time zero, glucose oxidase was added from the side arm of a Thunberg cuvette to the sample. As the enzyme reacted in the dark, short test flashes of 250 msec duration were given at regular intervals. These flashes were too short to cause an appreciable light-induced chl-a fluorescence quench but they were long enough to indicate the level of fluorescence at a particular point in time. As can be seen, this level changed in the course of the reaction with a rate of change being a function of PMS concentration.

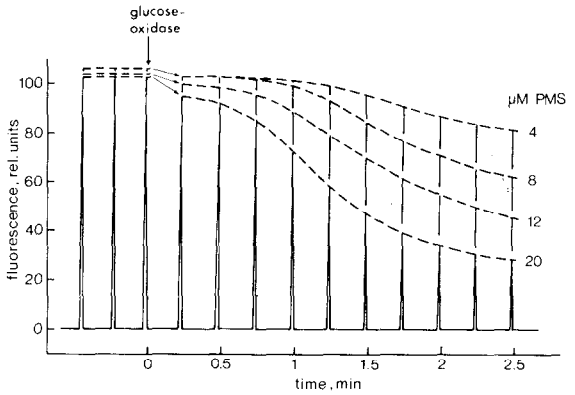

Fig.2. Time course of the fluorescence decrease upon reduction of PMS (dashed curves). The reaction mixture (see Methods) contained chloroplasts equivalent to 8 μg chlorophyll/ml and PMS concentrations as indicated. At zero time about 1 unit/ml of glucose oxidase was transfered from the side arm to the main flask of the Thunberg cuvette. At the time points indicated the fluorescence level was checked within time periods of 250 msec. Four different recorder traces refering to different PMS concentrations are superimposed.

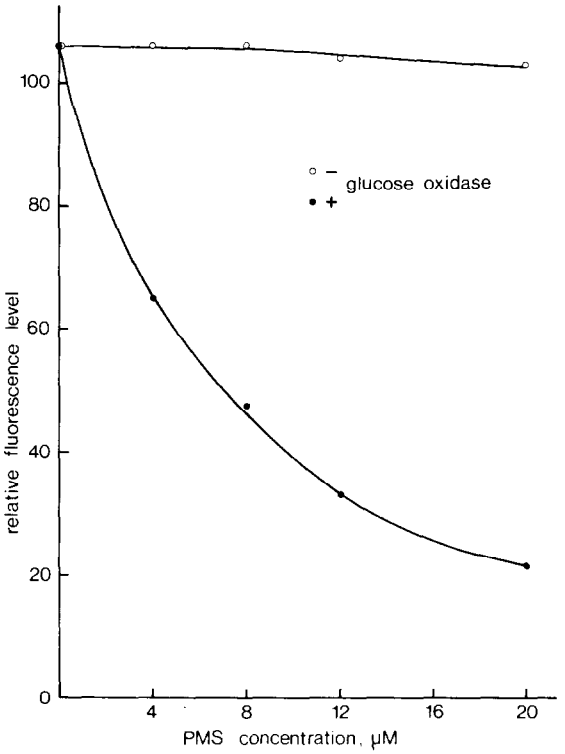

Fig.3. Effect of oxidised and reduced PMS on the fluorescence level. Chloroplasts equivalent to 8 μg chlorophyll/ml were incubated for 2 min in the reaction medium which additionally contained the PMS concentrations indicated. Thereafter 1 unit/ml glucose oxidase was added. (○) fluorescence level prior to enzyme addition, (●) fluorescence level 6 min after enzyme addition.

The final level which was obtained 6 min after the addition of glucose oxidase, is shown in fig.3 (closed circles). At the highest concentration of PMS used about 80% of the fluorescence was quenched. On the other hand the extent of fluorescence without enzyme was only slightly affected by PMS (fig.3, open circles) over the reaction time.

Related experiments using ascorbate instead of glucose/glucose oxidase yielded the same final fluorescence level (see fig.1). However, the time course (data not shown) revealed a rather fast quenching after mixing in the ascorbate followed by a slow further decrease.

Since ascorbate is a well known reductant for PMS it was supposed that under both conditions a reduction of PMS occured. As the time courses of the absorption at 388 nm in fig.4 show, the addition of glucose oxidase indeed resulted in a reduction of PMS. The absorption spectra for reduced PMS obtained after 5 min of incubation in a Thunberg cuvette with either 5 mM ascorbate or glucose oxidase were identical (not shown, but see ref. 7). The time lag at the beginning of the reaction is probably due to a competition between PMS and oxygen for the reductant.

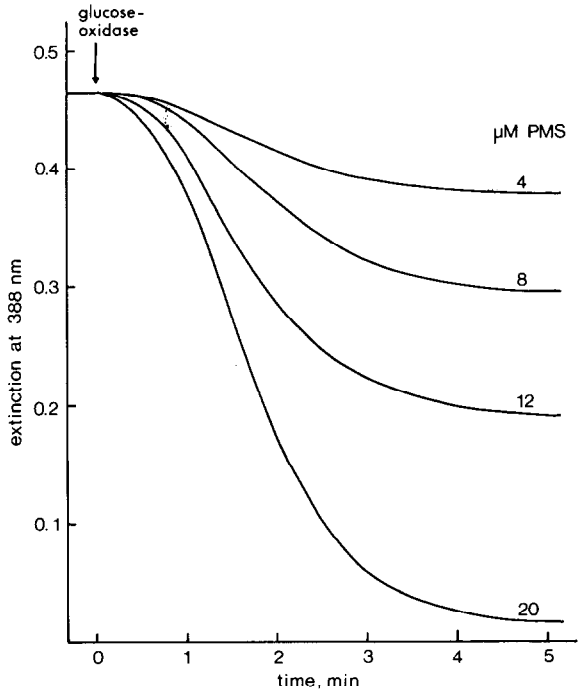

Fig.4. Reduction of PMS by the glucose oxidase couple. The reaction mixture (see methods) contained the concentrations of PMS as indicated. At zero time about 1 unit/ml of glucose oxidase was transfered from the side arm to the main flask of the Thunberg cuvette. Interruption of the trace due to mixing is not shown.

As was shown by Slovacek [8], PMS catalyses the reduction of oxygen by ascorbate. PMS is not completely reduced by ascorbate until the oxygen tension has markedly decreased. We measured the time course of the reduction of 20 μM PMS with 5 mM ascorbate and observed complete reduction of PMS after 5 min. The time course of the reduction had the same characteristics as the fluorescence decrease, as checked by the test flash method.

Mohanty et al. [3] did not find a marked decrease of chl-a fluorescence by the addition of ascorbate. Besides the different reaction conditions this discrepancy may be due to the fact that complete reduction of PMS by ascorbate needs a prolonged time of incubation. On the other hand Slovacek [8] showed a decreased chl-a fluorescence after the addition of ascorbate when PMS is present.

The above results suggest that reduced PMS is an effective quencher of chl-a fluorescence, far more effective than oxidized PMS [1]. The results should be regarded in the context of the light induced quenching of chl-a fluorescence [1–4] since we found that this quenching correlated with a light induced PMS reduction. A communication which presents new data concerning the light induced chl-a fluorescence quench is in preparation.

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